On the so-called "Sexual" Method of Spore-formation in the Disporic Bacteria.[1]

By

C. Clifford Dobell,

Fellow of Trinity College, Cambridge; Balfour Student in the University.

With Plate 13 and 3 Text-figures.

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INTRODUCTION.

Until the year 1902, no definite evidence regarding the sexuality of the Bacteria had been brought forward. It was generally supposed that the Bacteria constituted a group of very primitive organisms, in which the phenomenon of sex had not made its appearance. But with the publication of Schaudinn's remarkable researches on Bacillus bütschlii, it became apparent that such a supposition could not unreservedly be made. It appeared probable that—in B. bütschlii at least—a peculiar event in the life-history immediately preceding sporulation should be interpreted as

[1] I have already given a brief account of the work embodied in this paper to the Cambridge Philosophical Society, February 22nd, 1909.

representing a primitive or degenerate method of conjugation. If this interpretation is correct, then a fact of the utmost importance has been discovered—a fact of deep significance not merely as regards the affinities of the Bacteria, but also in relation to the general problem of sex.

For some years no confirmation of these observations was forthcoming; until, owing to the fortunate discovery of a new organism very like B. bütschlii, I was able—in 1907—to corroborate, in its essential points, the work of Schaudinn. Of the accuracy of Schaudinn's observations, I have myself no doubt whatever. But I cannot now regard his deductions from them as correct.

For various reasons, it seemed to me that the process which Schaudinn (1902) and others—including myself (1908)—were driven to regard as a form of conjugation, was, perhaps, capable of being interpreted in some other way. After much idle guessing, I concluded that the only method of throwing light upon the problem was to make a very careful study of sporulation in other Bacteria. Although so much has been written on this subject, it has been treated as a cytologic problem by but few investigators. And this is scarcely surprising, as the work has usually been done for medical purposes and upon organisms of very small size. One of the greatest difficulties which I encountered was that of obtaining species of Bacteria of sufficiently large size for exact observation. After many failures, I have now succeeded in following out the method of spore-formation in two large parasitic Bacteria which throw—as I believe—a considerable amount of light upon the "sexual" phenomena. It is my purpose in the following pages to describe these organisms and those stages in their life-histories which concern the problem under consideration. In a later part of the paper (p. 587) I shall point out the new point of view to which my researches have led me, and its consequences.

The descriptions which follow are based on a study of the organisms in their natural habitat. I have not isolated them in pure culture, as my object has been to study their ordinary ways of life, and not those occurring under artificial conditions.

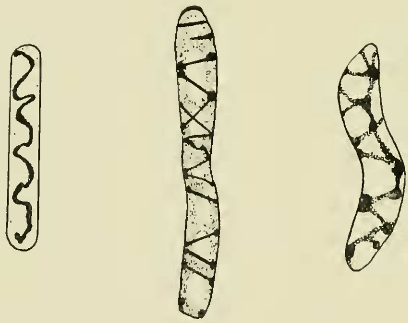
THE METHOD OF SPORE-FORMATION IN BACILLUS SPIROGYRA.

I have already (1908) given a brief description of the organism which I have named Bacillus spirogyra. I will here briefly recapitulate its main characteristics, and make a few additions and corrections to my original description.

B. spirogyra is a bacillus of large size—attaining a length of $12\,\mu$ by ca. $2\,\mu$—which I have found in the large intestine of frogs and toads, chiefly the latter. It is very uncommon. The most striking characteristic of this Bacillus is a spiral filament which runs from end to end. This filament varies a good deal in the way it is disposed. Sometimes it appears almost straight (Pl. 13, fig. 1), though as a rule it is in the form of an irregular spiral or zig-zag (fig. 2). Occasionally the filament is very much contorted (fig. 3). It is always deeply stained by nuclear stains—especially good differentiation being obtained with Giemsa's stain and Heidenhain's iron-hæmatoxylin. From its constancy and staining properties I think it is justifiable to regard this structure as a nucleus. Owing to the refractivity of the organism's pellicle, the filament cannot be made out with certainty in the living cell. I have never found any granular or other inclusions in the cells.

Spiral filaments such as this have already been described in several Bacteria—notably by Swellengrebel in Bacillus maximus buccalis (1906) and in Spirillum giganteum (1907). They have also been figured frequently in spirochæts. Several recent investigators have considered that these filaments do not really exist as independent structures; they suppose them to be merely an appearance due to the arrangement of the granules and protoplasmic alveoli of the cell [cf. Zettnow (1908), Hölling (1907), Guilliermond (1908), etc., and text-fig. A (3)]. That this is so in the case of B. spirogyra I cannot admit, nor, indeed, would anyone who had seen my preparations. I have already shown these to a number of persons, all of whom agree that such an interpretation is impossible. Not only are the varying disposition of the filament and its remarkable distinctness entirely against such an interpretation, but the appearance of burst forms (Pl. 13, figs. 4, 5) conclusively proves that the filament is a

TEXT-FIG. A.



1. 2. 3.

B. spirogyra (1). Sp. giganteum (2, 3),—2, after Swellengrebel (1907); 3, after Guilliermond (1908). In B. spirogyra there is a simple filament. In Sp. giganteum there is (according to Swellengrebel), a kind of irregular rodded spiral filament: according to Guilliermond the filament is falsely suggested by the arrangement of granules and cytoplasmic alveoli (3). The alveoli are, however, clearly shown by Swellengrebel (2) together with the filamentar structures.

solid body independent of the protoplasmic alveoli. In all carefully fixed and stained preparations the filament is as unmistakable as the nucleus of any other cell. In the case of Sp. giganteum, moreover, Swellengrebel has published figures which show both the filaments and the cytoplasmic alveoli in the same cell (see text-fig. A [2]).

I may add that I have been able to demonstrate the existence of a filament—independent of the cytoplasmic alveoli—in another Spirillum, which I hope to describe in a subsequent paper.

In my first account I stated that the filament in B. spirogyra became more twisted before division of the cell. This is not always the case; for I have found one or two individuals—though these are uncommon—which are dividing when the filament is in the form of an almost straight rod, (fig. 6).

Having said so much regarding the morphology of the organism, I will pass on to a description of the phenomena observed during spore-formation, which begins in the extreme posterior part of the host's rectum, but is not usually completed until the fæces have been discharged.

The first remarkable fact which I observed was that the individuals which were forming spores were little more than half the size of the ordinary individuals. After making measurements, I found that the average length of sporulating forms was about 5—6 μ, whilst that of ordinary individuals—selected at random—was roughly 9—10 μ. The reason for this was not difficult to discover. It appears that just before sporulation an ordinary transverse division of the cell takes place. Each daughter-cell then proceeds to form a spore without previously growing to the size of the ordinary individuals.

The details of spore-formation are as follows:—A large individual divides into two in the ordinary way—the chromatin filament being the first thing to divide (figs. 7, 8, 9). The daughter-cells produced by this division separate and undergo a certain amount of growth, though they never reach the size of the "parent" cell from which they were formed. Inside the cell the filament is seen to consist of comparatively few turns, and frequently displays a slight knob at one or both ends (fig. 10). One end of the filament now begins to enlarge—apparently at the expense of the rest of the spiral (figs. 11, 12)—so that a large nucleus-like mass is formed at one end of the cell (fig. 12). Up to this stage this body—

the spore rudiment—stains deep red, like chromatin, with Giemsa's stain. But a little later it changes its reaction, and is stained a bright blue (fig. 14). This, as I have already shown in B. flexilis, is owing to the formation of the spore membrane. As the membrane hardens the spore gradually stains less and less—until, finally, it refuses to stain at all (figs. 15, 16). At this stage the spore is fully formed, and the cell breaks up and liberates it more or less completely (fig. 16). It must be noted that a part of the filament persists outside the spore until quite a late stage, and breaks up with the rest of the cell (see figs. 14, 15, 16).

I occasionally found degenerating bacilli occurring together with those which appeared quite normal. Some of the forms encountered are shown in figs. 17, 18, and 19, and will require no further description.

A most remarkable—and, as I believe, important—variation in the method of forming spores was seen on a few occasions. The large original organism had failed to divide completely, and each of the small daughter individuals had developed spore rudiments whilst still attached to one another (fig. 13). [A discussion of these abnormal forms and their significance will be found on p. 587, et seq.]

The Method of Spore-formation in Bacterium lunula, n. sp.

Under the name Bacterium lunula I will here describe another micro-organism which I have found in the rectum of toads. It is, like the preceding form, very uncommon and of considerable size. It reaches a length of about 15 μ, though an average size is about 10 μ—12 μ.

The chief characteristic of this organism is its curved shape (fig. 20). The ordinary individuals appear to have a chromatic filament similar to that of B. spirogyra (cf. fig. 20), but not so clearly defined. I cannot state definitely that this is the normal condition, because on the few occasions on which I was fortunate enough to find these organisms, they were all beginning to sporulate. Hence, it is possible that the spiral arrangement of the chromatin appears only before spores are formed. I found very few individuals like that shown in fig. 20. Most of them had reached a more advanced stage in development.

The first stage in sporulation appears to be the constriction into two of a large form (fig. 21). The spiral is much more distinct at this stage. In some cases this constriction is so complete that two daughter-cells are formed, and separate as in B. spirogyra. Each daughter-cell, in a similar manner, then gives rise to a single spore (figs. 27, 28, 29). But in about an equal number of cases the constriction was incomplete or subsequently disappeared, and the large individual formed two terminal spores (figs. 22, 23, 25, 26), as in B. flexilis. The individuals which sporulate by the first method are therefore only about half the size of those which do so by the second method—these containing two spores, those one (cf. figs. 27 and 23). In the large individuals a trace of the original constriction was often to be seen (figs. 23, 26), and once or twice I saw forms in which it was still very pronounced (fig. 24).

Spore-formation appeared to take place just as in B. flexilis and B. spirogyra—that is to say, by an aggregation of chromatic material to form a spore-rudiment, and the subsequent formation of a spore membrane round this.

When the spore-rudiments are formed they resemble nuclei, and the organisms bear a very strong resemblance to a form recently described by Swellengrebel (1907 a) as Bacterium binucleatum. This bacterium contains two deeply-staining bodies, which careful micro-chemical tests lead Swellengrebel to suppose are nuclei. They appear to be constantly present, and divide in a curious manner. Another point of resemblance to B. lunula is the curved form which these organisms possess, thus causing them to resemble large Vibrios.

The Method of Spore-formation in B. bütschlii, B. flexilis, and other Disporic Bacteria.

In order that I may be able to discuss the general bearing of the foregoing observations on the central problem involved, I will here briefly summarise the descriptions which have been given of spore-formation in other disporic Bacteria.

First, I will recapitulate the process—the so-called "sexual" process—of spore-formation in B. bütschlii (Schaudinn, 1902) and B. flexilis (Dobell, 1908). The process is essentially the same in both (see text-fig. B, A, p. 589). A large individual (A 1), containing numerous chromatin granules, almost divides itself into two equal daughter-cells (A 2). The division is not completed, and subsequently all trace of it disappears (A 3). The granules now arrange themselves in the form of an irregular spiral, and at the same time begin to travel to opposite poles and mass themselves together to form the spore-rudiments (A 3, 4). Round these is formed a spore membrane, which gradually hardens and so gives rise to the completed resistant spore (A 5, 6). The remains of the cell, including a part of the chromatin spiral, perish.

Such is the process in these two Bacteria. If we search the literature bearing on the matter, we find that disporic forms are remarkably few—in many cases the existence of such forms is even denied, and it is stated that all Bacteria are monosporic. Several disporic Bactéria have, however, been observed; though in many cases it seems probable that the disporic individuals were exceptions, and monosporic individuals the rule. Prazmowski (1880) figured a large individual of a Clostridium sp. which possessed two terminal spores. Normally, however, but one spore is formed. In 1881 Kern described a large Bacillus from kephir, and proposed to name it Dispora caucasica, n. g., n. sp., on account of the fact that it regularly formed two terminal spores in each cell. De Bary (1884) in his text-book states that a single bacterial cell produces but a single spore:—

"Die seltene Ausnahme hiervon . . . dass nämlich zwei Sporen in einer Einzelzelle gebildet werden, kann in einem Uebersehen der Scheidewand zwischen zwei sporenbildenden Zellen ihren Grund haben." This view has been held by the majority of other writers. I may mention, however, that Ernst (1888) described and figured certain cases in Bacillus xerosis in which two spore-rudiments were present, without any signs of a septum between them; and that Frenzel (1892) found unmistakable cases of the existence of two spores in his very large "grüner Kaulquappenbacillus."[1] From the size of this organism, it seems to me unlikely that the septum—had it been present—would have been overlooked.

As far as I am aware, no other Bacteria which normally forms two spores have ever been described—and up to the present the process has been observed in detail in B. bütschlii and B. flexilis alone.

General Conclusions.

Schaudinn's interpretation of the phenomena observed in B. bütschlii was that the fusion of the two incompletely separated "daughter"-cells (text-fig. B, A 2, 3) represented a degenerate process of conjugation. A conjugation of this sort has been shown to take place in the yeasts (Schiönning, Hoffmeister, Janssen and Leblanc, Guilliermond);[2] in the diatom Achnanthes subsessilis (Karsten);[3] and in Protozoa, of which perhaps Actinosphærium furnishes the best example (Hertwig).[4] Conjugation of adjoining cells is

[1] This organism is of considerable interest in the present case, for it is another gigantic—occasionally disporic—form from Anura (tadpoles—probably of Bufo marinus). Frenzel describes the formation of the spore from a central nucleus-like body, but I think he was mistaken when he described the disporic forms as arising through the previous division of this body.

[2] See Barker, 'Annals of Botany,' vol. xv, 1901.

[3] See Klebahn, 'Arch. Protistenk,' Bd. 1, 1902.

[4] R. Hertwig, 'Abh. Akad. München,' xxix, 1898.

also known to occur in some filamentous Algæ—in Sphærozosma and Spondylosium (Desmidiaceæ),[1] and in Spirogyra, Zygnema, and other Conjugatæ. A comparable process has also been described in the Metazoa—in the parthenogenetic egg of Artemia.[2]

Now it appears to me, since observing the sporulation of B. spirogyra and B. lunula, that a much simpler interpretation of these phenomena in Bacteria is possible: namely, that we see here not a degenerate sexual process, but merely an abortive cell division. We have seen that a division takes place in B. spirogyra just before spore-formation, and that the daughter-cells which are so formed proceed to form spores without growing to their full size. We have only to suppose that this last cell division is not completed, to arrive at a result such as we see in B. bütschlii. There is, indeed, evidence to show that this last division may abort in B. spirogyra (cf. Pl. 13, fig. 13), so that individuals like the sporulating individuals of B. bütschlii result. My meaning will be made clearer by a glance at the accompanying figure (text-fig. B).

I mean to say that the disporic individuals which we find in B. bütschlii and B. flexilis are really double individuals—two individuals of the last generation of the life-cycle which have not been completely separated. It is a cell-division, not a sexual act, which has regressed. In Bacterium lunula, I believe, the regression has not gone so far, so that the last cell-division may be complete—giving rise, therefore, to the monosporic individuals—or abortive,—giving rise to the disporic individuals, as in B. bütschlii.

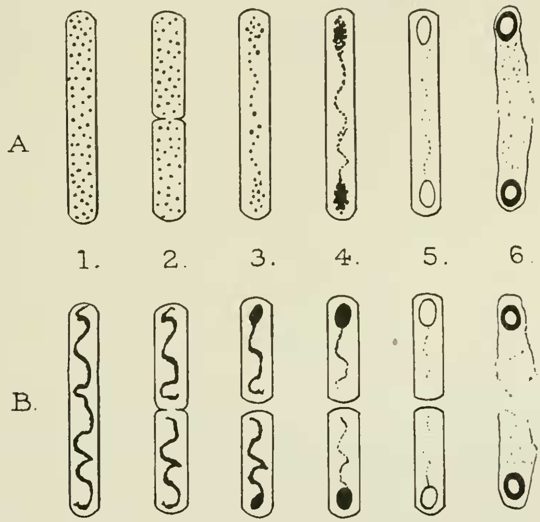
Schaudinn described abnormal monosporic individuals in B. bütschlii, and I also found them in B. flexilis. After I had reached the conclusions given above, I re-examined my old preparations of B. flexilis, and found several interesting abnormalities. In the first place, I found that the mono-

[1] De Bary, "Untersuchungen über die Familie der Conjugaten," Leipzig, 1858.

[2] Brauer, 'Arch. mik. Anat.,' 1893.

sporic individuals were nearly always very much smaller than those with two spores, but looked quite normal in other ways (figs. 31 and 32 are two instances). And I also found—though these were rare—forms which had remained almost completely divided right up to a late stage in sporulation (fig. 30). I thus felt that my original deductions from B.

Text-fig. B.



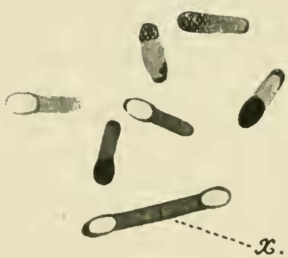
A. Spore-formation in Bacillus bütschlii or B. flexilis.
B. Spore-formation in B. spirogyra. (Diagrammatic.)

spirogyra and B. lunula were considerably strengthened. There is absolutely no evidence whatsoever that any process of conjugation takes place in B. spirogyra before the final cell division. Nor do I think any sexual significance would have been attributed to the phenomena in B. bütschlii if the sporulation of B. spirogyra had been previously known. The comparison with yeasts and algæ is, I believe, wholly misleading. I would also point out that the process in Actinosphærium is not in any case strictly comparable with that in B. bütschlii: for in this—assuming conjugation occurs—two zygotes result, whereas in that only one is formed.

The streaming of the granules observed so carefully by Schaudinn in B. bütschlii is due, I believe, merely to the fact that they have to travel to the poles of the cell to form the nucleus-like spore-rudiments.

Why the last division should abort in the disporic forms, must remain an open question. A possible explanation is that it is a process correlated with the parasitic manner of life. When the organisms are removed from their host in the

TEXT-FIG. C.



Bacilli (sp. incert.) from rectum of Bufo vulgaris; sporulating. x = a double individual. [Sublimate-alcohol, iron-alum hæmatoxylin; 2 mm. apochrom. x comp.-oc. 18. (× 2500).]

fæces, they would soon—in all probability—be dried, and hence induced to enter the resting state. And for purposes of dissemination, it is obvious that two spores are always better than one, so that the last division—albeit abortive—would double the number of individuals capable of sporulating.

In my preparations of B. spirogyra were many other Bacteria,—amongst them a small Bacillus which was also sporulating. The ordinary individuals formed a single terminal spore (text-fig. C) but here and there, double forms were to be found (x), which had spores placed at opposite poles. I find that similar forms have often been figured : for instance, Guilliermond (1908) gives several good examples (pl. 3, figs. 36—39, etc). In such organisms—which are obviously two still-attached individuals—the terminal position of the spores is remarkable when considered in relation to the disporic forms. Just as the latter have failed to form a complete septum, so have the former failed to separate after forming the septum.

The significance of the spiral configuration of the chromatin at a certain point in spore-formation in B. bütschlii and B. flexilis (text-fig. B, A 3, 4) still remains obscure. That it is connected with "conjugation" can scarcely be maintained, however, for as we have seen, it is permanently present in B. spirogyra. Perhaps a study of spore-formation in other forms may serve to throw some light upon its meaning. It may be noted, moreover, that Mencl (1905) has found similar arrangements of the chromation at certain periods in the life-history of some of the remarkable filamentous forms which he studied.

It seems to me that the parallel between the method of spore-formation in B. spirogyra and that of B. bütschlii is too close to be merely accidental. We have seen that the abnormal forms of the one are the same as the normal forms of the other. And when we consider further that in another case (B. lunula) spore-formation proceeds sometimes as in B. spirogyra, sometimes as in B. bütschlii, then it appears to me certain that the process of sporulation is really essentially the same in all these forms. If this is so, then there is but one alternative to my interpretation given above. It is, that conjugation takes place in all these forms, but that in B. spirogyra (always) and in B. lunula (sometimes) the conjugants separate before forming spores. As I have already said, there is absolutely no justification for such an assumption. The nuclear filament undergoes no changes which could be so interpreted—either in B. spirogyra or B. lunula. The significance of the "conjugation" of B. bütschlii and B. spirogyra is, to me, now quite clear: the last transverse division in the life-history has, for some reason, become abortive—division begins, progresses for a short way, and then regresses. But in this process two individuals are formed—each capable of sporulating—so that division is really completed physiologically, though not morphologically.

Now if my interpretation be correct—as I believe it is—it has some interesting results. In the first place, it has an important bearing upon the problem of the affinities of the Bacteria. Although there can, I think, be little doubt that the Bacteria are a very heterogeneous group, yet they present—as a whole—some well-defined features. And if we consider these, it seems to me that their affinities are not with the yeasts, as is often supposed, but in part with the Algæ and in part with other organisms. All recent work appears to me to show that the yeasts are really a low group of Fungi, which is properly placed in or near the Ascomycetes.

The similarity between the "conjugation" of the disporic Bacteria and the conjugation of yeasts furnished a strong piece of evidence in favour of the close kinship of these two groups—a piece of evidence which was, to me, wholly puzzling, but which is now, I think, shown to be false. My observations on B. spirogyra refute, I think, one of the strongest arguments in favour of the affinities of the Bacteria with the yeasts.[1]

There is one other point I must mention before I conclude, and that is with regard to the general phenomenon of sex. As is well known, Schaudinn—in his last papers (cf. Schaudinn, 1905)—advanced the view that sexuality is a fundamental property of living matter, having arisen when life itself arose—"so halte ich die Befruchtung für einen allen Lebewesen zukommenden Vorgang" (p. 34). This idea has been taken up and extended by Prowazek (1907), who has sought to show that sexuality is universal in the Protista, and that it is in some way correlated with the "diphasic nature" of protoplasm. How "sol"-phases and "gel"-phases of colloidal substances are connected (outside

[1] The name "Schizomycetes" is, I think, quite misleading when applied to the Bacteria, and should be dropped.

the imagination) with maleness and femaleness is—to me—quite a mystery. That there may be a connection, I cannot deny; but at present it seems to me that there is no more justification for such a view than there would be for correlating males and females with gas-engines and steam-engines.

I think Schaudinn's view was largely influenced by the fact that he found sexual phenomena not only in many Protozoa, but also—as he believed—in the Bacteria. If, then, it can be shown that the "sexual" process does not occur in Bacteria, one of the chief supports of the view vanishes. And—as I believe—the observations recorded in the beginning of this paper show that—in the disporic forms at least—no real sexual process occurs. For my own part, I do not think the evidence supports the hypothesis that sexuality is a fundamental property of living matter. And certainly I see no evidence in favour of such a hypothesis derived from the Bacteria.

I confess there is still one unaccountable case of "sexuality" described in Bacteria—that of B. sporonema (Schaudinn, 1903). This organism, however, stands alone. Perhaps future work will give us an explanation of the meaning of its "conjugation." At all events, it is premature to generalise from this single case at present. I hope my own researches—which are still in progress—on the Bacteria and allied forms may shed some further light upon this most interesting phenomenon.

Zoological Laboratory,
Cambridge;
February, 1909.

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DESCRIPTION OF PLATE 13,

Illustrating Mr. C. Clifford Dobell's paper "On the so-called 'Sexual' Method of Spore-formation in the Disporic Bacteria."

[All figures are from preparations fixed with formalin and stained by a modification of Giemsa's method. Drawings made under a Zeiss 2 mm. homog. oil immersion apochromatic, comp.-oc. 12. Magnification ca. 2000 diameters.]

Figs. 1—19. Bacillus spirogyra.

Fig. 1.—An individual with an almost straight nuclear filament.

Fig. 2.—An individual with well-marked spiral or zig-zag filament.

Fig. 3.—An individual in which the filament is very greatly contorted.

Figs. 4, 5.—Two burst individuals, in which the filament is clearly seen to be a solid independent structure lying in the cell.

Fig. 6.—Division of an individual with a straight filament.

Figs. 7—16. Stages in spore-formation.

Figs. 7, 8, 9.—Stages in division of a large individual into two.

Fig. 10.—Small individual formed by division of a large one—about to form a spore.

Fig. 11.—A similar organism at a later stage. One end of the filament is much enlarged, forming the spore-rudiment.

Fig. 12.—A later stage, in which the spore-rudiment is further developed.

Fig. 13.—An abnormal case, in which a large individual has not completely divided, and in which each of the (still attached) daughter-individuals contains a spore-rudiment similar to Fig. 11.

Fig. 14.—Stage succeeding Fig. 12. The spore-membrane has just been formed, and is stained a bright blue.

Figs. 15, 16.—Later stages—completion of spore-formation. In Fig. 16 the spore is fully formed, and the remains of the cell are breaking up.

Figs. 17, 18, 19.—Degenerate forms.

Figs. 20—29. *Bacterium lunula*, n.sp.

Fig. 20.—Ordinary individual before sporulation.

Fig. 21.—Individual dividing into two, preparatory to forming spores.

Figs. 22, 23, 25, 26.—Successive stages in the development of a disporic (double) individual.

Fig. 24.—An individual, almost divided into two—each half containing a spore-rudiment.

Figs. 27, 28, 29.—Stages in development of a spore in a monosporic individual.

Figs. 30—32. *Bacillus flexilis*.

Fig. 30.—An abnormal individual, which—at a late stage in spore-formation—is almost completely divided into two.

Figs. 31, 32 —Stages in development of (small) abnormal monosporic individuals.